AMENDMENT

Kindly amend the application, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows.

IN THE CLAIMS:

Kindly amend the claims, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, to read as follows:

1. (Currently Amended) A method for ~~releasing~~ extracting a soluble or membrane associated intracellular recombinant protein of interest (POI) from a bacterial, yeast or fungal cell, the POI being released from the bacterial, yeast or fungal cell, comprising the steps of:

(a) providing a bacterial, yeast or fungal cell comprising a soluble or membrane associated intracellular recombinant POI;

(b) releasing the recombinant POI from the cell by contacting the cell with a membrane extracting composition comprising a quarternary ammonium compound at a concentration of between 0.05% to 0.6% by weight ~~for between 4 to 48 hours~~, under conditions sufficient for the release of the recombinant POI in a soluble form; and,

(c) recovering the recombinant POI from the membrane extracting composition ~~in which the released recombinant POI has a specific activity higher than when it has been extracted by mechanical means~~.

2. (Original) The method according to claim 1, wherein the quarternary ammonium compound is selected from the group consisting of Lauroyl Trimethyl Ammonium Bromide (LTAB), Myristyl Trimethyl Ammonium Chloride (MTAC), CetylTrimethyl Ammonium Chloride (CTAC), Cetrimide, Cetyl Trimethyl Ammonium Bromide (CTAB), Stearoyl Trimethyl Ammonium Chloride (STAC), Stearoyl Trimethyl Ammonium Bromide (STAB), Benzalkonium Chloride (alkyldimethylbenzylammonium chloride), N-Cetylpyridinium Bromide (N-Hexadecylpyridinium bromide), N-Cetylpyridinium Chloride (N-Hexadecylpyridinium chloride), Benzyl Dimcthyl Tetradecyl Ammonium Chloride, Benzyl

Dimethyl Hexadecyl Ammonium Chloride and a combination of any two or more thereof.

3. (Cancelled)

4. (Previously Presented) The method according to claim 1, wherein the membrane extracting composition comprises from about 0.1% to about 0.5% by weight of the quarternary ammonium compound.

5. (Original) The method according to claim 4, wherein the membrane extracting composition comprises from about 0.2% to about 0.45% by weight of the quarternary ammonium compound.

6. (Original) The method according to claim 5, wherein the membrane extracting composition comprises about 0.4% by weight of the quarternary ammonium compound.

7. (Original) The method according to claim 1, wherein the cell is contacted with the membrane extracting composition at temperatures from about 4°C to 40°C.

8. (Original) The method according to claim 7, wherein the cell is contacted with the membrane extracting composition at temperatures from about 20°C to about 30°C.

9. (Original) The method according to claim 8, wherein the cell is contacted with the membrane extracting composition at temperatures from about 25°C.

10. (Previously Presented) The method according to claim 1, wherein the cell is contacted with the membrane extracting composition at a pH of from about 2.0 to about 11.0.

11. (Previously Presented) The method according to claim 10, wherein the cell is contacted with the membrane extracting composition at a pH of from about 5.0 to about 7.0.

12. (Previously Presented) The method according to claim 11, wherein the cell is contacted with the membrane extracting composition at a pH of from about 6.3.

13-14. (Cancelled)

15. (Original) The method according to claim 1, wherein the cell is a transformed cell.

16. (Original) The method according to claim 1, wherein the cell is transformed with a nucleic acid encoding the POI.

17. (Previously Presented) The method according to claim 1, wherein the intracellular recombinant POI is produced by recombinant DNA techniques.

18. (Withdrawn) The method according to claim 1, wherein the recombinant POI is an interleukin 1 receptor antagonist (IL-1ra) enzyme.

19. (Withdrawn) The method according to claim 1, wherein the recombinant POI is a glucan lyase enzyme.

20. (Withdrawn) The method according to claim 19, wherein the yield of glucan lyase is 1 g/litre or more.

21. (Withdrawn) The method according to claim 19, wherein the yield of glucan lyase is 3.5 g/litre or more.

22. (Previously Presented) The method according to claim 1, wherein the recombinant POI is a hexose oxiadase (HOX) enzyme.

23. (Currently Amended) The method according to claim 22, wherein the hexose oxidase (HOX) enzyme comprises the amino acid sequence set out in SEQ ID No ~~22~~ 23.

24. (Previously Presented) The method according to claim 22, wherein the hexose oxidase (HOX) enzyme is encoded by a nucleotide sequence set out in SEQ ID No 22.

25. (Previously Presented) The method according to claim 22, wherein the hexose oxidase (HOX) enzyme is encoded by a nucleotide sequence capable of hybridising to the nucleotide sequence set out in SEQ ID No 22 or a sequence complementary to the hybridisable sequence.

26. (Currently Amended) A method for screening for mutated cells or transformed cells producing elevated levels of a soluble or membrane associated intracellular recombinant POI comprising the steps of:

(a) growing the mutated cells at 30°C;
(b) incubating the mutated cells or transformed cells with the membrane extracting composition comprising a quarternary ammonium compound at a concentration of between 0.05% to 0.6% by weight ~~for between 4 to 48 hours~~,
(c) recovering the cell free medium; and
(d) screening the cell free medium for elevated levels of the intracellular recombinant POI;

such that the presence of the intracellular recombinant POI in the cell free medium is indicative that the intracellular recombinant POI has been released.

27. (Previously Presented) A membrane extracting composition suitable for releasing a soluble or membrane associated intracellular recombinant POI, wherein the composition is contacted with the cell under the following conditions:

(a) a percentage by weight of quarternary ammonium compound from about 0.05% to about 0.6%;
(b) a pH of from about 2.0 to about 11.0; and
(c) a temperature of from about 4°C to about 40°C;

such that the intracellular recombinant POI substantially free of contaminating proteins is

obtained.

28. (Currently Amended) A method of using a membrane extracting composition comprising a quarternary ammonium compound to ~~selectively release~~ extract a soluble or membrane associated intracellular recombinant POI from a bacterial, yeast or fungal cell, the POI being released from the bacterial, yeast or fungal cell, in which the membrane extracting composition comprses a quarternary ammonium compound at a concentration of between 0.05% to 0.6% by weight and is contacted with the bacterial, yeast or fungal cell ~~for between 4 to 48 hours~~ and in which the released recombinant POI is recovered from the membrane extracting composition.

29. (Previously Presented) A hexose oxidase (HOX) enzyme producible by a method according to claim 1, wherein the hexose oxidase (HOX) enzyme is encoded by a nucleotide sequence set out in SEQ ID No 22, or a sequence complementary to the hybridisable sequence, and wherein the nucleotide sequence is synthesised by the oligonucleotides as set out in SEQ ID Nos 2-22.

30. (Withdrawn) A POI as defined in claim 1, wherein the POI is released in a substantially non-glycoslyated form from a eukaryotic host organism.

31. (Withdrawn) A substantially non-glycosylated POI released from a eukaryotic host organism.

32. (Withdrawn) A substantially non-glycosylated POI released from a eukaryotic host organism, wherein the POI is released by the method of claim 1.